MILLIPORE

# MultiScreen® Filter Plate with Ultracel®-10 Membrane: Enzymatic activity recovery

## Application note AN2011EN00

MultiScreen Ultracel-10 filter plate provides a new method for high throughput sample preparation. The ultrafiltration-based filter plate is designed for automation-compatible sample purification, concentration and desalting of biological solutions. The 96-well MultiScreen filter plate incorporates MultiScreen Ultracel-10 ultrafiltration membrane (10,000 nominal molecular weight limit regenerated cellulose) for ultra low-binding, high recovery results. It is designed for use with centrifugation and is compatible with standard microtiter plates, instrumentation, and liquid handling equipment.

This study demonstrates the use of MultiScreen Ultracel-10 plate for concentration of alkaline phosphatase without loss of biological activity and with high reproducibility across the plate.

### Method:

#### Reagents

- Milli-Q® water
- Diethanolamine Sigma D-8885
- Magnesium Chloride Hexahydrate, Sigma M-2670
- Phosphate substrate 104-0 Sigma Diagnostics INC
- Alkaline Phosphatase Sigma P3877

**Reagent A**: 1.0 M Diethanolamine Buffer, 0.50mM Magnesium Chloride (prepared fresh).
**Reagent B**: 5 ml of 150mM p-Nitrophenyl Phosphate Solution (PNPP) (prepared fresh).
**Reagent C**:50ml of Phosphatase Alkaline Enzyme Solution of 0.1 – 0.2 Units/ml.
Prepared fresh in cold reagent A.

#### Equipment and materials

- Millipore MultiScreen Ultracel-10 filter plate
- V-bottom microtiter plate (Greiner catalog number 651201)
- Clear flat bottom collection plates (Costar® 9017, Corning Inc.)
- Jouan CR312 centrifuge equipped with swinging bucket rotor with plate carriers
- Mettler Toledo balance
- SpectraMax Plus plate reader with software 4.1
- Diethanolamine Assay Protocol, Sigma EC 3.1.3.1

#### Protocol

1. Place MultiScreen Ultracel-10 plate on top of 96 well collection plate (Greiner 651201).
2. Add 200 ul of Alkaline phosphatase solution to each well of MultiScreen Ultracel-10 plate.
3. Centrifuge at 2500 x g for 5, 10, 20 and 40 min.
4. Measure the volume of the retentate.

5. Transfer 6.45 uL of the retentate from each well a clear flat bottom Costar plate containing reagents A and B in the following ratio:

| | Test | Blank |
|---|---|---|
| **Reagent A** | 174.2 µl | 180µl |
| **Reagent B (substrate)** | 19.35µl | 19.35µl |
| **Reagent C (enzyme)** | 6.45µl | 0.0 µl |

6. Read plate immediately on SpectraMax plate reader at 405-nm wavelength, acquiring readings at 0, 1, 2, 3, 4 and 5 minutes at 37°C.
7. Calculate Alkaline Phosphatase activity by obtaining first the $\Delta A_{405}$/min using the maximum linear rate for both the test and the blank, multiplied by the volume of the assay and dilution factor, and divided by the extinction coefficient for p-Nitrophenol at 405nm times the volume of enzyme used.

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405}/\text{min Test} - \Delta A_{405}/\text{min Blank})\ (0.2)\ (1)}{(18.5)\ (0.00645)}$$

Where:
0.2 = Volume (in Milliliters) of assay
1 = Dilution factor
18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm
0.00645 = volume (in milliliters) of enzyme used

**Alkaline Phosphatase Unit definition**: One Unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 9.8 at 37°C.

## Results

Ultrafiltration is used in practically every biotechnology process[1]. There are multiple papers describing its use in the purification protocols for animal, plant, fungal and bacterial proteins[2-5]. While other protein purification techniques such as reverse phase or ion exchange chromatography may be challenging to scale-up, ultrafiltration is easily scaleable process and so is beneficial to the biotech industry when high-throughput of product is required[6].

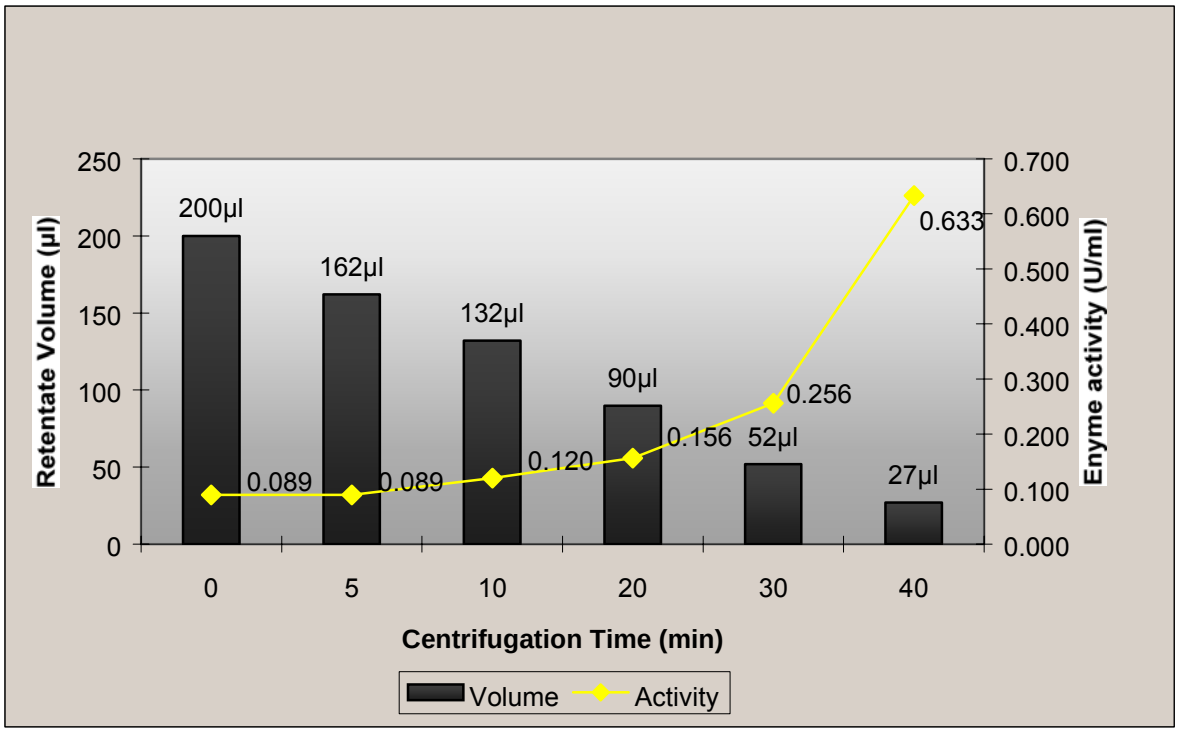
MultiScreen Ultracel-10 96-well filtration plate incorporates 10,000 nominal molecular weight limit regenerated cellulose ultrafiltration membrane for ultra low-binding, high recovery results. It is designed for use with centrifugation and is compatible with standard multiwell plates, instrumentation, and liquid handling equipment.

In most protein purification processes, increase in protein purity and concentration and preservation of biological activity are of equal importance. We have shown before that high level of protein concentration can be achieved reproducibly in MultiScreen Ultracel-10 plates (PC1025EN00: Protein Retention, Recovery, Volume Recovery, and Guidelines for Concentration and Desalting). In this study, we have investigated preservation of of alkaline phosphatase activity during concentration from a dilute solution using MultiScreen Ultracel-10 plate in high throughput format.

Starting solution contained 0.089 Units/ml of alkaline phosphatase. It was aliquoted into 96 wells of MultiScreen Ultracel-10 plate and concentrated by centifugation down to 20-30 uL in 40 minutes. Figure 1 shows reduction of the phosphatase solution volume and increase of enzymatic activity throughout the concentration process.

The results demonstrate that alkaline phosphatase solution can be concentrated 7.4 fold with parallel 7.1 fold increase in phosphatase activity, with 95.87% yield. High yield of activity was achieved in a simple centrifugation procedure, without any pre-treatment or passivation of the ultrafiltration membrane.

**Figure 1.** Alkaline Phosphatase solution volume reduction and enzymatic activity increase during centrifugation in MultiScreen Ultracel-10 plate. Each data point represents an average volume and phosphatase activity for 16 wells.

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